A Study of the Taxonomic Composition of Bacterial Populations in Fresh-Water Lakes

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Division of Laboratories
ONTARIO WATER RESOURCES COMMISSION

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Abstract

Three lakes representing low, moderate and high levels of nutrients were sampled in June 1970. The total plate count was determined for each sample and from these plates isolated colonies were picked and identified. The bacterial taxa isolated from the lakes and the non random manner of their variation was determined. An index of nutrient levels in lake water based on the numbers of Pseudomonadaceae, Achromobacteriaceae and Enterobacteriaceae isolated was described.

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Introduction

This study investigated the distribution of bacterial genera from different nutrient environments. Three lakes from the Trent River System were chosen to represent conditions of eutrophy, mesotrophy and oligotrophy. The classification of the lakes as characteristic of these three states was determined by Christie (5). On the basis of the morpho-edaphic index (total dissolved solids in parts per million per average depth in feet), he described Kushog Lake as oligotrophic, Balsam Lake as mesotrophic and Clear Lake as eutrophic.

Oligotrophic lakes are characterized by clear water, a low level of nutrients, and sparse plant growth. Deeper waters remain well supplied with oxygen throughout the year. Eutrophic lakes contain high nutrient levels and their waters are frequently turbid from dense growths of phytoplankton. The growth of aquatic vegetation is heavy. When water circulation is restricted, the decomposition of organic matter depletes deeper areas of the lake of oxygen. Mesotrophic lakes reflect conditions midway between those of eutrophic and oligotrophic waters (7).

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E. A. Bennett (1) compared the total plate count populations of Lake Ontario and Lake Superior. The Pseudomonas, Flavobacterium and Acinetobacter were the most commonly occurring genera in Lake Ontario and represented 56% of the isolated organisms. The Enterobacteriaceae family accounted for 18% of the isolates from this lake. In Lake Superior, 74% of the organisms fell into the Bacillus, Pseudomonas and Acinetobacter groups. The different proportions of genera were attributed to the difference in nutrient level between the lakes. The Lake Superior waters were oligotrophic. Lake Ontario had a more nutrient enriched environment tending towards mesotrophy or eutrophy.

We expected the generic composition and generic frequency of the total plate count population to vary with the quantity of nutrients. The bacterial populations of Kushog, Balsam and Clear Lakes were characterized in an attempt to demonstrate and describe this variation.

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Methods

Water samples were collected from Kushog, Balsam and Clear Lakes in three periods of five consecutive days each. Only one lake was sampled during each period. The samples were taken from Balsam Lake on June 8 through June 12; from Kushog Lake on June 13 through June 17 and from Clear Lake on June 22 through June 26, 1970. The sampling of the three lakes was carried out by the Division of Sanitary Engineering in connection with the Recreational Water Pollution Control Program.

On Balsam and Kushog Lakes, the sampling points were determined by superimposing a grid on maps of the lakes. A single grid unit had an area of approximately one fifth of a square mile. The Balsam Lake sampling points were arranged on the vertices of non-adjacent grid units. In Kushog Lake, because of its narrowness, sampling stations were appointed to diagonally opposite vertices of each grid unit. The use of a grid allowed an even distribution of points over the entire lake surface. Twenty sampling locations were determined on both Balsam and Kushog Lakes. The distributions of sampling stations are shown in Figures 1 and 2. On Clear Lake, sample points

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were designated by the Division of Sanitary Engineering. Ten stations, shown in Figure 3, were sampled on this lake.

The water samples, of 250 mls. volumes, were taken at one metre depths. As the samples were collected, they were placed on ice and were stored this way until analyzed. The water temperature was determined at the time of sampling.

The analyses were done for total plate count by the method of membrane filtration as described by Bennett and Jones (1 and 8). The culture medium for total plate counts was m-Plate Count Broth (Difco) buffered with 0.1% di-Potassium phosphate. The incubation was at 20°C in a humid atmosphere for 48 hours. The use of black membrane filters (Millipore) facilitated the counting of colonies. The analyses were performed in mobile field laboratories, at or near each lake, and within 12 hours of sample collection.

Once the total plate count densities had been determined for each day's samples, the total plate count plates were packed with ice and transported to the main Toronto laboratories. The transportation took approximately four hours. The plates were unpacked and refrigerated on arrival. The next day, colonies were picked from one total plate count plate from each of the sampling locations. The colonies were chosen by visually dividing the plate into

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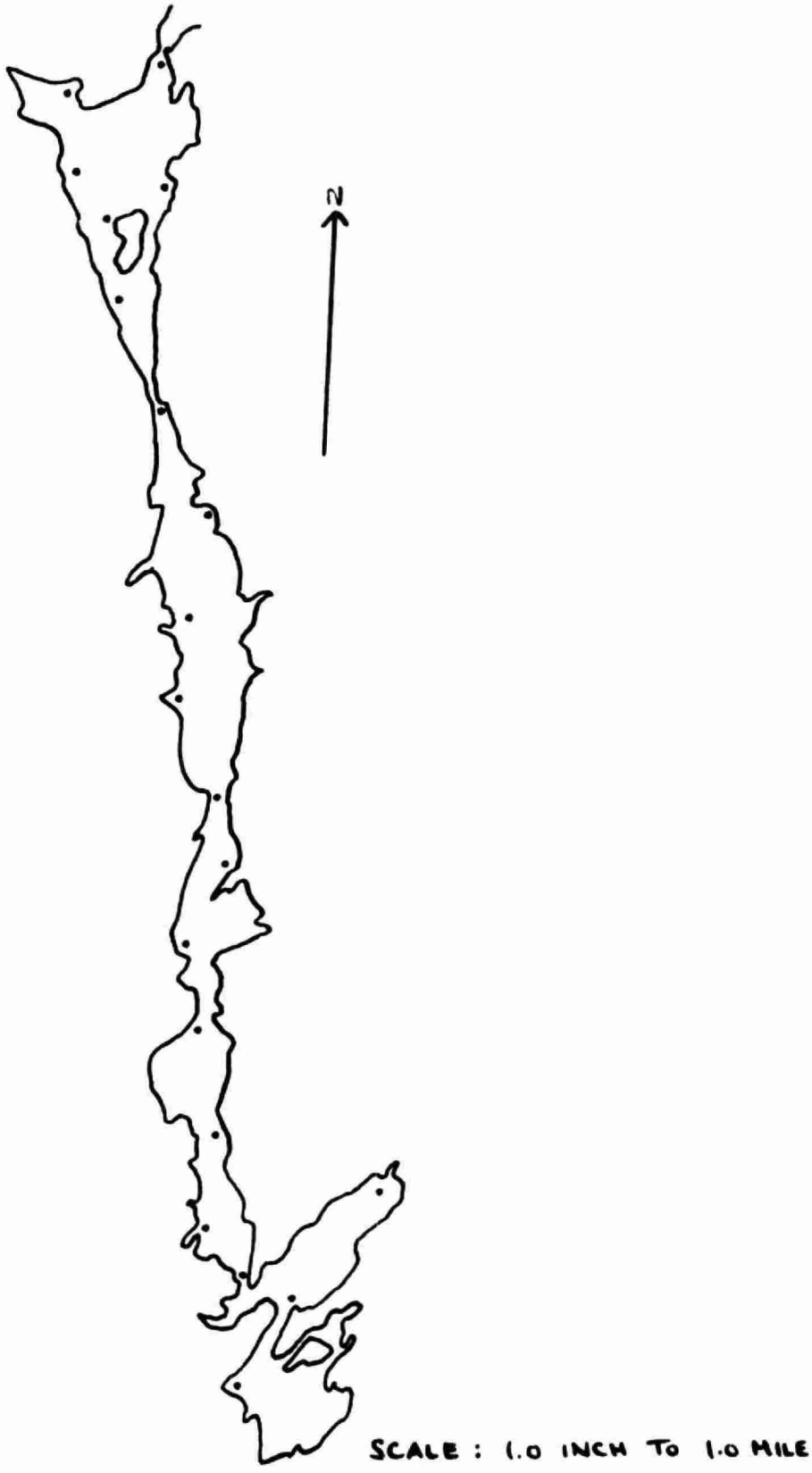

FIGURE 1: The distribution of sampling locations on Kushog Lake

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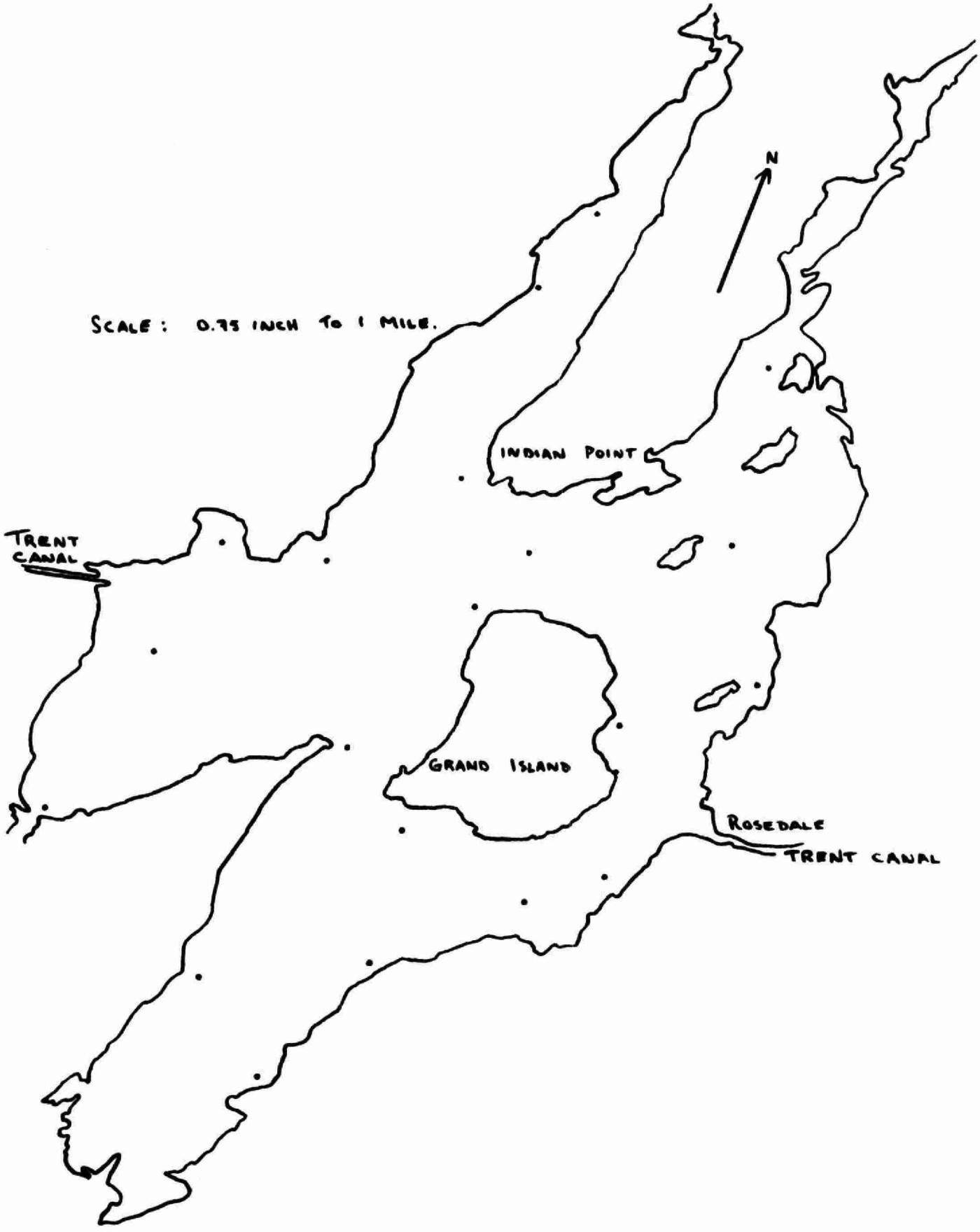

FIGURE 2: The distribution of sampling locations on Balsam Lake

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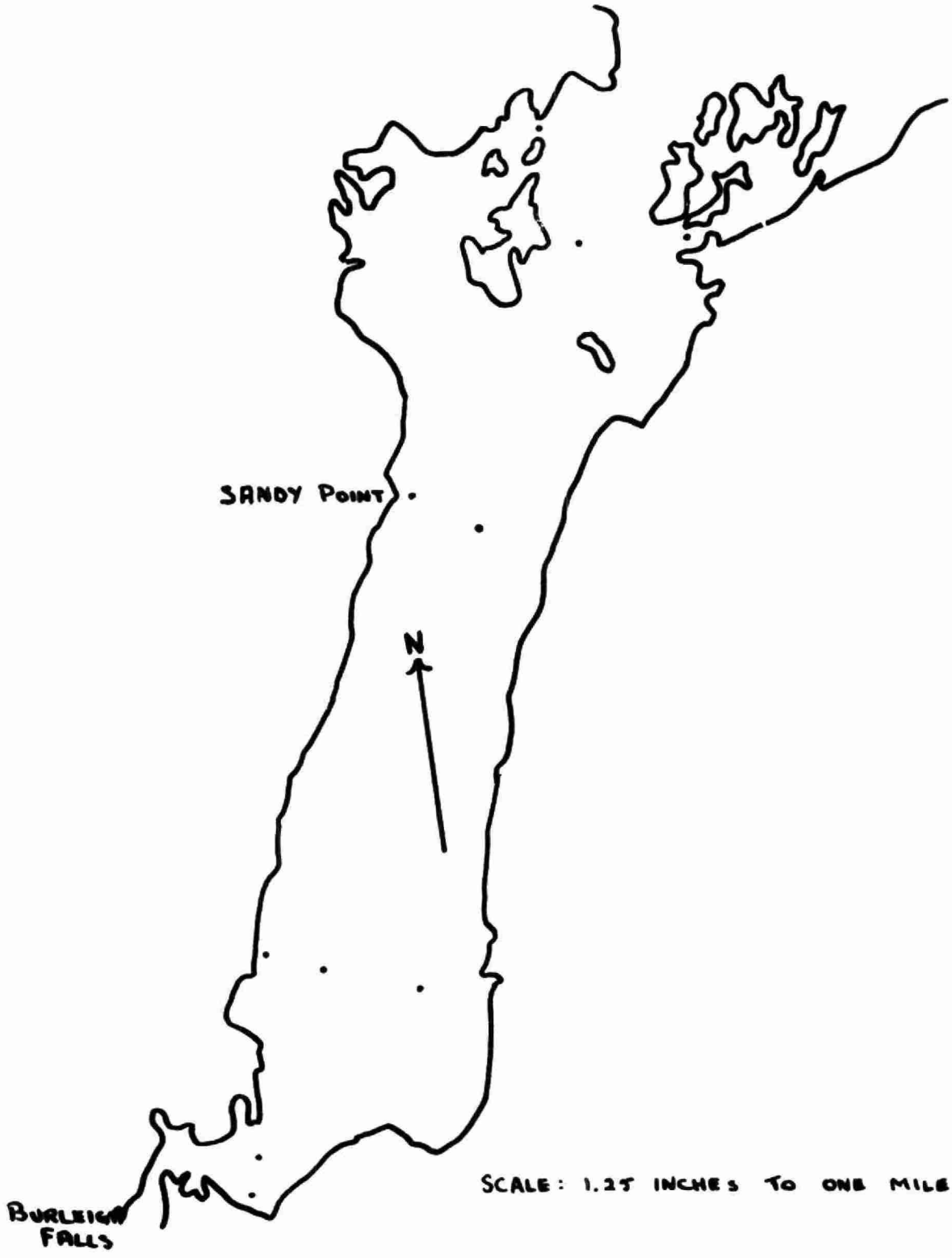

FIGURE 3: The distribution of sampling locations on Clear Lake

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sectors containing ten colonies and picking all ten colonies from that sector. This procedure allowed the relative proportion of the different genera represented on the plate to be maintained. The isolated colonies were inoculated onto Trypticase-soy (Difco) agar slants and incubated at 20°C until growth appeared (usually three to five days). The cultures were then stored in the refrigerator for periods varying from two to six weeks. The storage of cultures was necessary because 2500 isolates were collected over the three week sampling period.

The growth from the refrigerated slants was recovered and purified by streaking the inoculum on Trypticase-soy (Difco) agar plates. The incubation was at 20°C for five days. The isolated colonies were then streaked onto fresh Trypticase-soy agar slants. The slants were incubated at 20°C for 48 hours at which time Gram stains were done to check culture purity. The Gram stain reaction and the microscopic morphology of pure cultures were recorded.

The biochemical characterization of the cultures included tests for the presence of catalase (slide method), oxidase, motility (hanging drop method with two passages through Trypticase-soy broth (Difco)), acid and gas production in Phenol-Red Dextrose Broth (Difco), reaction in

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the glucose Oxidation-Fermentation medium of Hugh and Leifson (Difco), and growth on MacConkey agar (Difco). The test reactions were read after 48 hours of incubation at 20°C. The Oxidation-Fermentation medium, if negative after 48 hours, was reincubated for another 72 hours and checked at 24 hour intervals. Spore stains were performed on Gram positive bacteria and on those Gram negative bacteria which failed to grow on MacConkey agar. A detailed description of test reagents, media and procedures has been reported by Bennett (1).

The identification of cultures was made on the basis of schemes presented by Cowan and Steel (6) as outlined by Bennett (1). The scheme provided an identification to the generic level except for members of the Enterobacteriaceae family. In this study, it was not feasible to determine a more detailed classification of the Enterobacteriaceae and their description was left at the familiar level.

The chi-square test of homogeneity was carried out on genera frequency data using the formula:

$$\text{chi-square} = \frac{\sum (x - \bar{x})^2}{\bar{x}}$$

where x was the number of isolates within each taxa for each lake and $\bar{x}$ was the average number of isolates across the lakes.

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The calculated values were compared with critical chi-square values for N-1 degrees of freedom, tabulated by Rohlf and Sokal (8). The geometric mean values of total plate counts were calculated as:

$$G.M. = \text{antilog} \frac{\sum x}{N}$$

where x was the common logarithm of the count and N, the number of observations.

An analysis of variance was carried out to test the homogeneity of the geometric means. Table I presents the method of data treatment for this analysis. The F ratios calculated were compared with the critical values of the F distribution as tabulated by Rohlf and Sokal (8).

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TABLE I - Outline of the Analysis of Variance performed on total plate count data.

Variation	Degrees of freedom (d.f.)	Sum of Squares (S.S.)	Mean squares (M.S.)
Total	N-1	$\sum (x - m)^2$	
Between stations	R-1	$\sum (\bar{r} - m)^2$	S.S./d.f.
Within stations	remaining d.f.	remaining S.S.	S.S./d.f.

N = total number of counts determined

R = total number of stations sampled

x = the logarithmically transformed total plate count

$m = \frac{\sum x}{N}$

$\bar{r}$ = the average total plate count for each station sampled.

The F ratio is the quotient:

$$\frac{\text{M.S. (Between stations)}}{\text{M.S. (Within stations)}}$$

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Results and Discussion

The total plate counts from each lake were grouped according to the sampling locations and a geometric mean was calculated for each group. The mean total plate counts from stations on Kushog Lake ranged from 4,600 to 20,000 organisms per 100 mls. This range of values represented higher densities than could be demonstrated at Balsam Lake stations and at all but two Clear Lake stations. The analysis of variance performed on logarithmically transformed data from Kushog Lake gave a non significant F ratio. This indicated that the variation of counts between the stations was less than the variation occurring from day to day within each station. One mean value for the lake was considered as representative of all stations. Its calculated value was 10,000 organisms per 100 mls.

The mean densities of bacteria at Balsam Lake stations ranged from 200 to 3,000 organisms per 100 mls. The majority of stations (15 of the 20 sampled) showed lower mean values than those obtained from Clear Lake where counts varied from 1610 to 10,800 organisms per 100 mls. The analysis of variance performed on data from

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both Clear and Balsam Lakes indicated that the station mean values demonstrated greater variation in comparison with each other than was demonstrated within the stations from day to day. Single mean values representative of the entire lakes were not calculated for this reason.

The total plate counts varied in a manner independent of the nutrient level. Highest counts came from the oligotrophic waters of Kushog Lake and the lowest counts from mesotrophic Balsam Lake. Conditions of mesotrophy and eutrophy resulted in a high degree of variation in total counts indicating that increased nutrient levels stimulated density fluctuations.

The distribution of total plate count isolates throughout the lakes was presented in the form of frequency distributions. The number of organisms within each taxonomic group from each station were summed over the five days on which samples had been collected. The frequency with which these total numbers of isolations occurred were plotted as a function of them.

The frequency distributions of genera isolated from Kushog Lake are shown in Figure 4. The histograms suggested a Poisson distribution of isolates,

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characterized by the decreasing probability of high numbers of events (in this case, high numbers of isolations). An extreme example of this type of distribution was shown by the Acinetobacter and Achromobacter genera. The incidence of no isolation of these groups exceeded the incidence of their isolation. The distributions of the Pseudomonas, Arthrobacter-Corynebacterium and the Alcaligenes were more reliable approximations of the Poisson distribution because of the lower frequency of zero isolations. The isolation frequency of Enterobacteriaceae increased as the number of isolations rose. This atypical distribution was attributed to the high proportion of total isolates represented by the Enterobacteriaceae. In half of the stations sampled they accounted for 80% or greater, of all isolations. Seventy percent of isolates from the entire lake fell within this family.

The genera isolation frequencies for Balsam Lake appear in Figures 5A and 5B. The less frequent incidence of zero isolations improved the reliability of the Acinetobacter, Achromobacter and Arthrobacter-Corynebacterium distributions. The genus Alcaligenes displayed a smaller range of isolation numbers than in Kushog Lake but its distribution remained in good approximation of the Poisson.

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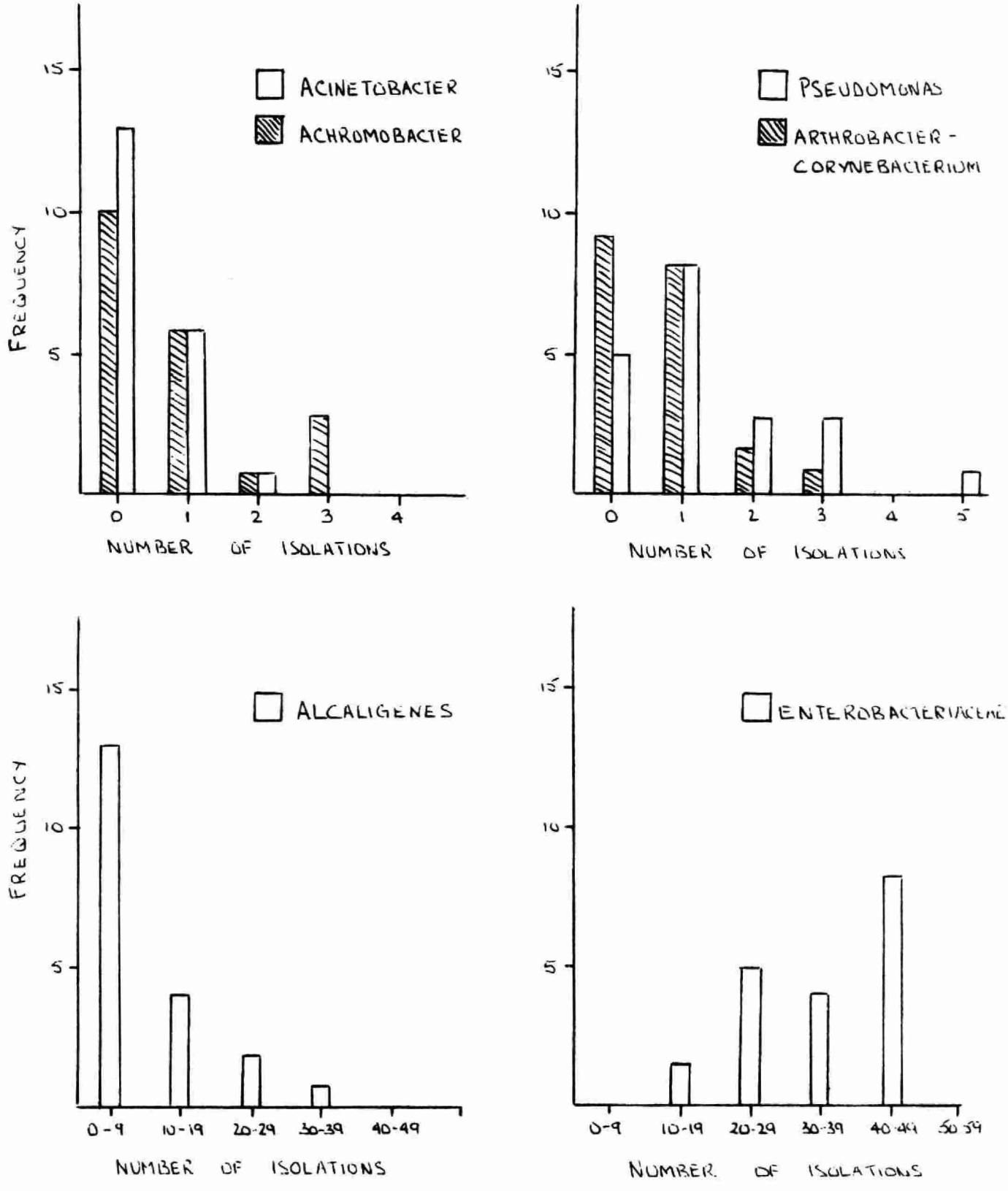

FIGURE 4: The Frequency Distributions of Taxa Isolated from Kushog Lake

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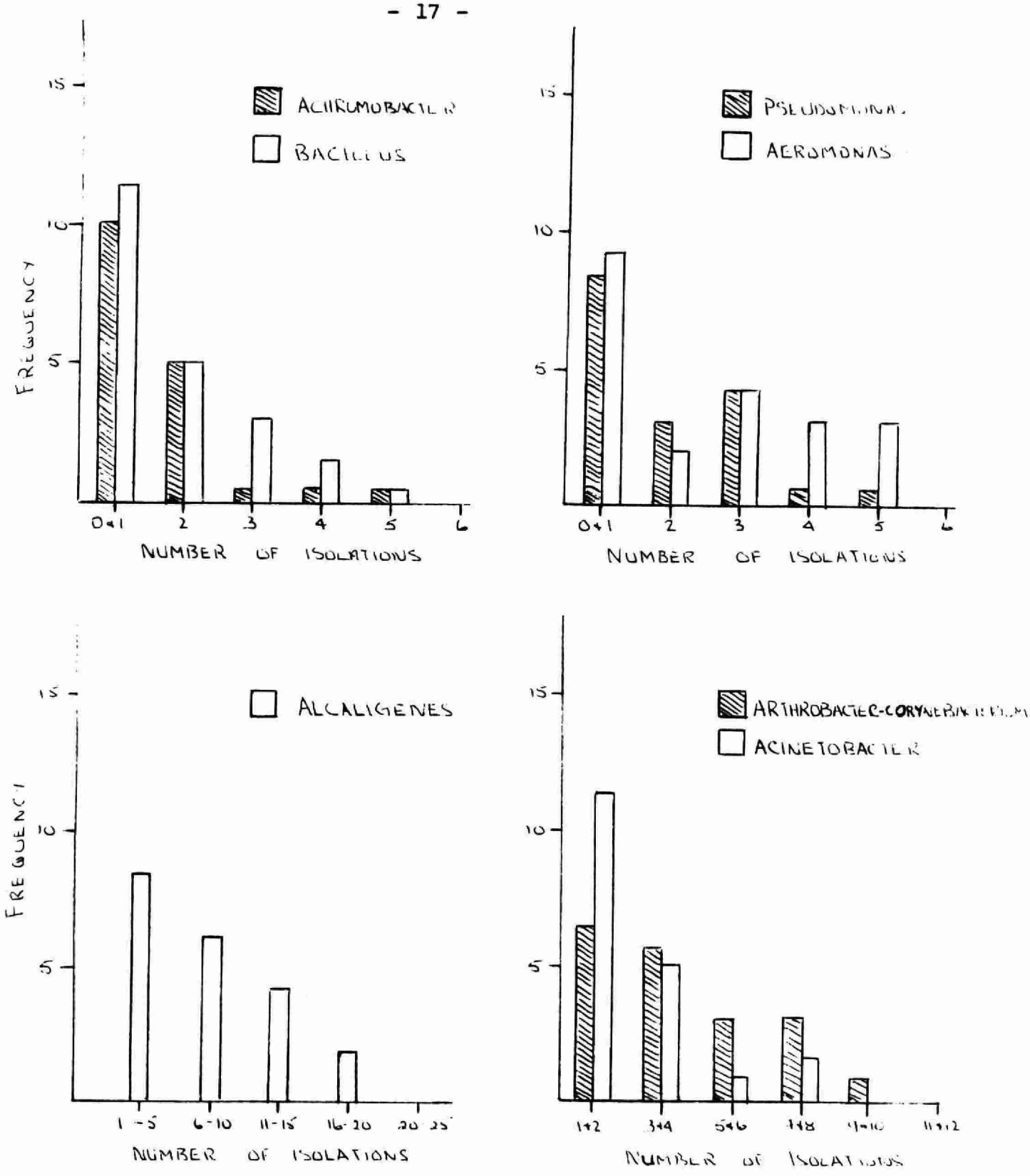

FIGURE 5A: The Frequency Distributions of Taxa Isolated from Balsam Lake

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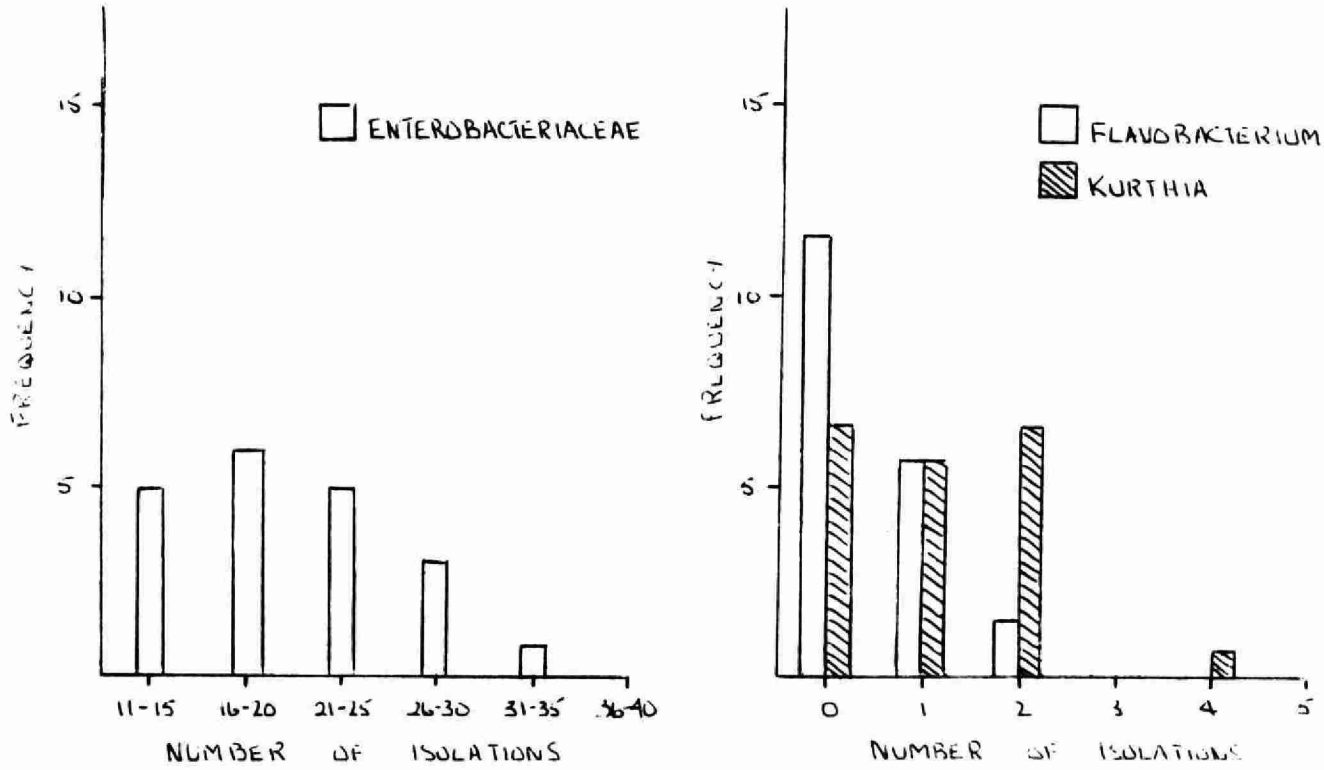

FIGURE 5B: The Frequency Distribution of Taxa Isolated from Balsam Lake (continued)

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The distribution of the genus Pseudomonas showed an increased frequency of higher numbers of isolations. The effects of this were not sufficiently large to distort the downward trend of the histogram. As in Kushog Lake, the Enterobacteriaceae were distributed in an atypical fashion in Balsam Lake. Their distribution seemed to approximate a normal distribution with a mean number of isolations of sixteen to twenty organisms. The Bacillus and Aeromonas frequency distributions closely approximated the typical Poisson. The histograms describing isolation frequencies of the Kurthia and Flavobacterium genera displayed a Poisson tendency but were less reliable due to the high frequency of zero isolations.

The distribution frequencies of isolates from Clear Lake are presented in Figure 6. Only ten locations were sampled on this lake, whereas Balsam and Kushog Lakes were sampled at twenty points. The non-descript nature of the distribution histograms for the Enterobacteriaceae and the Acinetobacter was attributred to this fact. The decreasing isolation frequencies of the Pseudomonas, Achromobacter, Aeromonas and Arthrobacter-Corynebacterium suggested a Poisson distribution of these genera. The genus Alcaligenes demonstrated decreasing frequencies of

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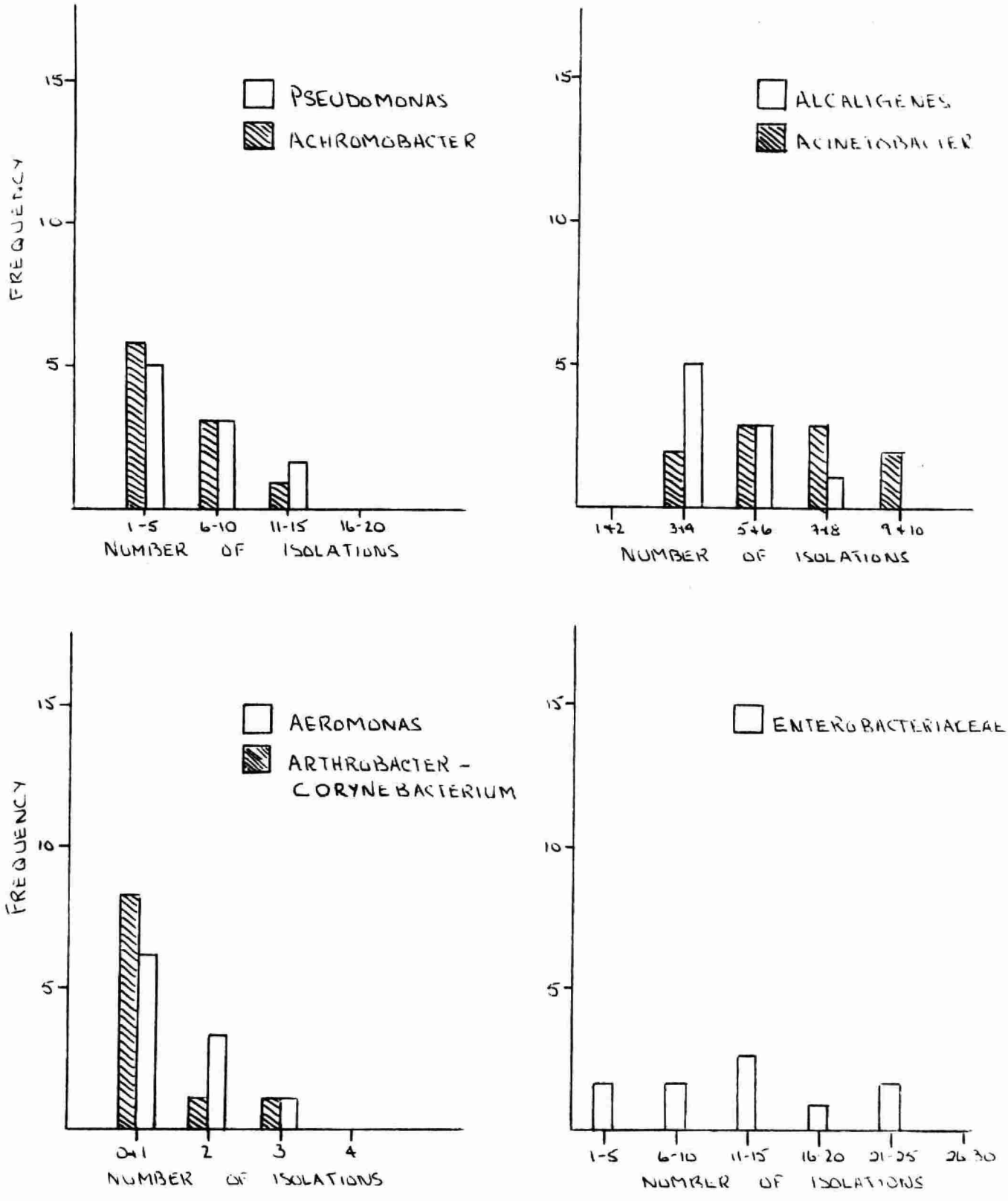

FIGURE 6: The Frequency Distribution of Taxa Isolated from Clear Lake

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isolation, but, since low numbers of this organism did not occur, a Poisson distribution was not indicated.

The approximate Poisson distribution of the total plate count isolates within the lakes indicated that the isolation of an organism was an independent event. Specifically, the occurrence of a bacterial genus at one station was not influenced by the presence of organisms at other stations. This pattern of distribution was expected on the basis of a random sampling of evenly distributed populations within each lake.

The total isolation frequency of each taxonomic group of organisms from each lake was prepared. The frequency values for Clear Lake were multiplied by two to compensate for the fact that only ten stations were sampled on this lake while twenty locations were sampled on Kushog and Balsam Lakes. To determine if the isolation frequencies varied in a random fashion across the lakes, the chi-square test of homogeneity was carried out. The results are presented in Table II.

The frequency of occurrence of Acinetobacter and Achromobacter increased as the nutrient level of the water rose from oligotrophy in Kushog Lake to eutrophy in Clear Lake. The significant value of the chi-square

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TABLE II - The number of organisms within taxa isolated from Kushog, Balsam and Clear Lakes, and the chi-square values calculated for each taxon.

	Number of Isolations			
	Kushog Lake	Balsam Lake	Clear Lake	Chi-square value
Achromobacter	17	33	100	77.6*
Acinetobacter	8	55	122	106.4*
Aeromonas	3	46	20	40.8*
Alcaligenes	174	162	100	28.2*
Arthrobacter-Corynebacterium	15	80	22	170.2*
Bacillus	1	26	22	22.1*
Flavobacterium		11	8	
Kurthia	2	24	10	20.7*
Micrococcus	1	6	4	3.4
Pseudomonas	31	47	130	81.5*
Enterobacteriaceae	700	408	264	216.6*

* Values significant at the 99% level of confidence for degrees of freedom = N-1 = 2.

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statistic indicated that these changing frequencies of isolation were not due to chance. The Aeromonas, Arthrobacter-Corynebacterium and the Kurthia genera also presented significantly variable distributions and demonstrated a preference for the mesotrophic waters of Balsam Lake. The isolations of the Alcaligenes and Pseudomonas groups occurred with consistent frequencies in the oligotrophic and mesotrophic lakes. In eutrophic Clear Lake however the Alcaligenes isolations became less frequent while isolations of the Pseudomonas increased. A significant chi-square value obtained for both these genera indicated that the fluctuation of their isolation frequencies was not random. The Bacillus species were isolated with equal frequency from Balsam and Clear Lakes where conditions of mesotrophy and eutrophy occurred. The oligotrophic nature of Kushog Lake reduced the frequency of Bacillus isolates sufficiently to produce a significant chi-square value. The Enterobacteriaceae family was distributed in a significantly non-random manner. The isolation frequencies of this family declined steadily from high levels in oligotrophic Kushog Lake to low levels in eutrophic Clear Lake. The genus Micrococcus, distributed randomly across the lakes, displayed isolation

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frequencies independent of nutrient level.

The temperature differences between the lakes were insufficient to account for the significant variation of genera isolation frequencies across the lakes. The average temperatures of Kushog, Balsam and Clear Lakes were 21.0°C, 20.9°C and 19.0°C respectively. The range of temperatures over the three lakes was from 17.0°C to 22.5°C. This 5.5°C range of variation was too narrow to explain the significantly different distribution of isolates.

The total Coliform count has been used as an index of water quality and distributions of total plate count isolates grouped according to Coliform densities have been described (1,2,8). The Coliform counts were divided into ranges and the isolated genera grouped within them according to the count recorded for the water sample from which the isolates came. The Acinetobacter displayed a significantly increasing frequency of isolation as the level of Coliform densities rose. It was suggested that Acinetobacter, a naturally occurring organism in water and soil, was responding to increased nutrient levels. The distribution of Acinetobacter across Kushog, Balsam and Clear Lakes confirmed this hypothesis since its isolations

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were increasing frequent as the level of nutrient enrichment rose.

The preference of the Enterobacteriaceae for the oligotrophic conditions of Kushog Lake supported Bennett's finding that a significantly higher frequency of these organisms occurred in less polluted waters (1). However, the author demonstrated previously that the Enterobacteriaceae were increasingly isolated from waters of high Coliform densities (8). The mean Coliform counts in Kushog Lake were high in comparison with those of the other two lakes, ranging from 45.3 to 604.0 organisms per 100 mls.* The high number of Enterobacteriaceae was perhaps in response to high Coliform counts rather than an oligotrophic nutrient level. In Balsam and Clear Lakes, the isolation frequency of the Enterobacteriaceae displayed an independence of Coliform counts. Balsam Lake exhibited a range of Coliform mean counts from 1.0 to 24.3 organisms per 100 mls. where in Clear Lake the means ranged from 51.0 to 162.2 organisms per 100 mls., yet the Enterobacteriaceae were one and one-half times more frequently isolated from Balsam Lake than from Clear Lake. The Enterobacteriaceae therefore did

* The total Coliform count data was provided by the Bacteriology Branch studies for the Recreational Water Pollution Control Program (4).

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demonstrate a preference for clean waters, in agreement with Bennett's result (1), and in a manner independent of the Coliform counts.

Bennett (2) demonstrated that the Aeromonas genus was significantly, more frequently isolated from polluted water characterized by high Coliform levels. In the present study, this genus was isolated most frequently from the low Coliform dense and mesotrophic waters of Balsam Lake. The preference of the Aeromonas for moderate nutrient levels was unaffected by low Coliform levels.

The Flavobacterium (1,8) and the Bacillus (8) have shown significant tendencies of increased isolations in clean waters. From Kushog Lake however very low numbers of these organisms were isolated. They occurred with a consistent and higher frequency in the more enriched waters of Balsam and Clear Lake. The number of isolations showed no tendency to increase in Balsam Lake where Coliform counts were lowest. The genus Pseudomonas has also shown preference for unpolluted waters (8). The frequency of Pseudomonas isolations in this study steadily increased as the nutrient levels rose in spite of the low Coliform counts occurring in the mesotrophic waters.

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The distribution of total plate count bacteria isolated from Kushog, Balsam and Clear Lakes is presented in Table III in terms of the three taxonomic families most frequently represented. The genera were grouped into families on the basis of classifications presented in Bergey's Manual (3). The Pseudomonadaceae family included the Aeromonas and Pseudomonas, and the Achromobacteriaceae family included Achromobacter, Acinetobacter, Alcaligenes and Flavobacterium. The chi-square test of homogeneity was carried out to determine if the familiar distribution of isolates was random.

Members of the family Pseudomonadaceae were less frequently isolated from the lakes than were the Achromobacteriaceae and Enterobacteriaceae. The largest number of isolates fell within the Enterobacteriaceae except in Clear Lake where Achromobacteriaceae were the most commonly occurring family. For all three families, the calculated chi-square values were significant which indicated that the fluctuations in isolation frequencies were not due to chance. The Pseudomonadaceae and Achromobacteriaceae occurred with low frequency in Kushog Lake and became more frequent in the mesotrophic and eutrophic conditions of Balsam and Clear Lakes.

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The changing proportions of the Pseudomonadaceae, Achromobacteriaceae and Enterobacteriacea suggested that these three families could be used as an index of nutrient enrichment in water. A quotient value describing their interrelationship was calculated:

$$\text{INDEX VALUE} = \frac{\text{NUMBER OF ISOLATIONS OF PSEUDOMONADACEAE AND ACHROMOBACTERIACEAE}}{\text{NUMBER OF ISOLATIONS OF ENTEROBACTERIACEAE}}.$$

The value of this index for oligotrophic waters (Kushog Lake) was 0.33, for mesotrophic waters (Balsam Lake) 0.87, and for eutrophic waters (Clear Lake) 1.82. Further investigations of the proportional distribution of Pseudomonadaceae, Achromobacteriaceae and Enterobacteriaceae are needed to ascertain the reliability and significance of this index. In the present study, the index values increased in magnitude by an order of three as the nutrient environment changed from oligotrophy through mesotrophy to eutrophy. It was considered an appropriate and potentially useful indicator of the different nutrient levels of lake water.

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TABLE III - The number of organisms within the Pseudomonadaceae, Achromobacteriaceae and Enterobacteriaceae families isolated from Kushog, Balsam and Clear Lakes, and the chi-square values calculated for each family.

	Number of Isolations			
	Kushog Lake	Balsam Lake	Clear Lake	Chi-square value
Pseudomonadaceae	34	93	150	136.9*
Achromobacteriaceae	199	261	330	80.9*
Enterobacteriaceae	700	408	264	215.8*

* Value significant at the 99% level of confidence for degrees of freedom = N-1 = 2.

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Summary and Recommendations

1. The total plate counts varied in a manner independent of the nutrient level of the lakes. The highest counts were from the oligotrophic waters of Kushog Lake and the lowest counts from mesotrophic Balsam Lake.

2. The generic composition of the bacterial population depended upon the nutrional environment from which the population was sampled. The Acinetobacter, Achromobacter, Alcaligenes and Pseudomonas showed highest isolation frequencies in the nutrient enriched waters of Clear Lake. The moderate nutrient levels of Balsam Lake were preferred by the Aeromonas, Kurthia and Arthrobacter-Corynebacterium genera. The Bacillus occurred with consistent frequency in Clear and Balsam Lakes but was recovered only infrequently from oligotrophic Kushog Lake. The Enterobacteriaceae family was isolated in greatest numbers from Kushog Lake.

3. When the isolated bacteria were grouped according to taxonomic families rather than genera, the Achromobacteriaceae and Pseudomonadaceae were shown to increase as Enterobacteriaceae decreased. The proportion of Achromobacteriaceae and Pseudomonadaceae isolations to Enterobacteriaceae isolations reflected numerically the

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different nutrient levels. The proportional distribution of these three families should be investigated in other lakes of defined nutrient levels as their relationship was a useful indicator of the conditions of oligotrophy, mesotrophy or eutrophy.

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Acknowledgements

I wish to thank Miss Ingrid Wiechmann and Mrs. Claudette Walters for their excellent technical assistance. Thanks are extended to the field crews of the Bacteriology Branch and District Engineers Branch for the collection of samples and determination of total plate counts.

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